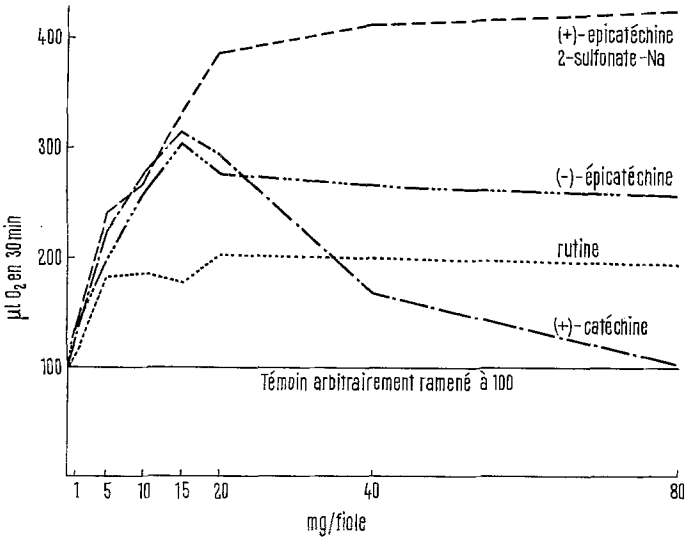

Activité des flavonoïdes étudiés sur l'enzyme respiratoire.

Il est intéressant de noter, en outre, que l'augmentation d'activité enzymatique constatée pour certains produits semble liée à l'apparition d'un pigment coloré, plus ou moins abondant suivant les cas.

Conclusion. Les faits expérimentaux ainsi mis en évidence nous semblent justifier un prolongement de cette étude orienté dans deux directions principales:

1. Tenter d'élucider in vitro la relation existant entre le flavonoïde et l'activité enzymatique mitochondriale, et notamment préciser la structure et le rôle du pigment coloré mis en évidence.

2. Confirmer in vivo les résultats obtenus en administrant à l'animal les substances à étudier.

Summary. The effect of 4 different flavonoids on the respiratory activity of cytochrome oxidase in rat liver homogenate was measured in vitro using the Warburg apparatus. The results show that 3 compounds of the catechin group are especially active.

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Intracellular Localization and Heterogeneity of Alkaline Phosphatase of Yoshida Ascites Sarcoma

Multiplicity in size has been shown for alkaline phosphatase (orthophosphoric monoester phosphohydrolase EC 3.1.3.1) from human placenta[1], human serum[2], rat liver[3] and a myeloblastic tumour[4]. A large alkaline phosphatase (excluded from Sephadex G-200) has been reported in serum[2], and in an animal tumour[4]. This association was further examined in the animal tumour Yoshida ascites sarcoma and the present paper reports the findings of this study.

The tumour, obtained from the Chester Beatty Institute, London, was kept in continuous passage in random bred Wistar rats by the injection of 1.0 ml of ascitic fluid i.p. Rats were sacrificed 7 days after implant, when they yielded 10–20 ml of ascitic fluid, containing 2–4 g of cells (wet weight), per rat. The cells were washed in normal saline, and homogenized in an all glass homogeniser (Potter-Elvejem) in hypotonic buffer (*Tris* HCl 10 m*M* pH 7.7). For differential centrifugation the cells were similarly ruptured in normal saline (SCHNEIDER and HOGEBOOM[5]). Then the precipitates were solubilized as follows: a) The nuclear fraction by homogenizing in hypotonic *Tris* HCl (preceeded by 2 isotonic saline washes); b) and c) mitochondria and microsome as above; d) the soluble supernatant was left in normal saline.

Gel filtration profiles of the cell fractions were obtained using a 50×2 cm column of Sephadex G-200. Alkaline phosphatase activity was measured using the method described by DUNNE et al.[2] and is expressed in IU: cytochrome oxidase activity, used as a marker for the differential centrifugation procedure, was measured by the manometric assay of SCHNEIDER and POTTER[6] and total nitrogen was determined by the micro-Kjeldahl method modified by CONWAY[7].

Yoshida ascites sarcoma has low alkaline phosphatase activity, 0.01 IU/mg *N*, as compared with 0.41 IU/mg *N* for another animal tumour, Shay Chloroma (DUNNE et al.[4]). The cell-free ascitic fluid contained a relatively large amount of enzyme.

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The intracellular distribution of alkaline phosphatase is given in the Table. The enzyme was located mainly in the soluble supernatant fraction (30,000 × *g* for 7 h), with some activity in the nuclear and microsomal fractions. Although a similar finding has been reported by CHAUDHARY et al.[8], for the enzyme of Tribolium confusum, the localisation of alkaline phosphatase does seem to depend on the source. The enzyme in mammalian tissues occurs predominantly in the microsomal fraction (MORTON[9]; VAES and JACQUES[10]); in He-La cells it occurs in the nuclear fraction (COX and GRIFFIN[11]), and in the liver of tumour bearing animals the enzyme is located in the nuclear and microsomal fractions (NAKATA et al.[12]). The variations in techniques of homogenization and isolation of cell fractions used by different investigators could also be a factor contributing to the apparent variation in intracellular localisation of the enzyme.

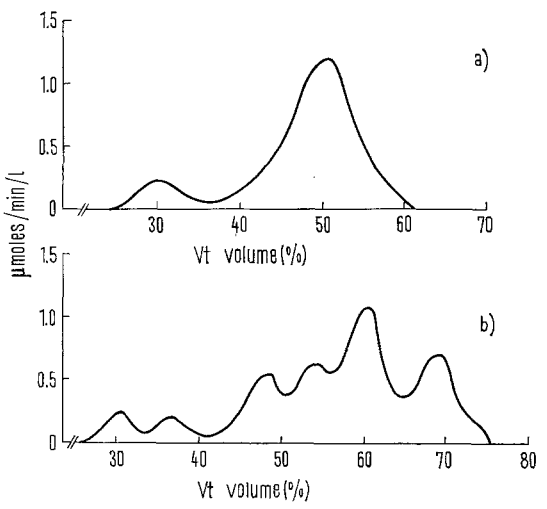
The gel filtration profiles (Sephadex G-200) of the homogenate and soluble supernatant fraction (Figures a and b) show 2 peaks of enzyme activity in the nuclear fraction and 6 peaks of activity in the soluble supernatant. The first or excluded enzyme peak of both fractions is eluted with the void volume of the column as is a phosphatase found in human tumour extracts (FENNELLY et al.[13]).

The exclusion of this enzyme from Sephadex G-200 suggests that it has a minimum molecular weight of 600,000 (ANDREWS[14]). This may be a further evidence of a possible association of a macromolecular form of alkaline phosphatase with neoplasia (DUNNE et al.[2,4]). The elution point of the sixth peak of enzyme activity of the soluble supernatant fraction would indicate it has a molecular weight of 8 to 10,000 (ANDREWS[14]) which is very small for alkaline phosphatase. The elution point of the other 5 enzyme peaks suggest that they may result from polymerization of this sixth peak enzyme or alternatively from depolymerization of the excluded enzyme. The alkaline phosphatase of *E. coli* is a dimer of molecular weight 86,000 but the monomers of alkaline phosphatase with molecular weight 43,000 are enzymatically inactive (SCHLESINGER[15]). BUTTERWORTH[16] has suggested that the alkaline phosphatase of human urine originates from the kidney by a process involving the dissociation into subunits of the kidney enzyme; the depolymerized kidney enzyme, however, was, like the *E. coli* monomer, inactive. If depolymerization results in loss of enzyme activity, the activity of the small sixth peak enzyme could be due to the possible reaggregation of enzyme molecules in the collection tubes following gel filtration and before the enzyme assay. The reaggregation alternatively could have been induced by the substrate or the pH of the assay mixture. Multiplicity in size has been shown for alkaline phosphatase from other sources – human placental enzyme (GHOSH and FISHMAN[1]) and the enzyme of the soluble fraction of rat liver (KOSCHNITZ et al.[3]).

Intracellular localization of alkaline phosphatase in Yoshida ascites sarcoma cells

Fraction	Total activity	Total activity (%)	Specific activity
Nuclei	0.053 (104.3)	21.2 (17.6)	0.002 (21.3)
Mitochondria	0.0 (456.06)	0.0 (77.3)	0.0 (574.3)
Microsomes	0.048 (30.0)	19.4 (5.07)	0.007 (23.3)
Soluble supernatant	0.148 (0.0)	59.4 (0.0)	0.125 (0.0)

Total alkaline phosphatase activity expressed in μmoles of *p*-nitrophenyl phosphate hydrolyzed/min; specific activity expressed in μmoles/min/mgN. Cytochrome oxidase activity, used as a marker for the differential centrifugation procedure, is shown in brackets and is expressed in μl O_2/10 min (total activity) and μl O_2/10 min/mgN (specific activity).



Gel filtration profile (Sephadex G-200) of a) homogenate and b) soluble supernatant fraction of Yoshida ascites sarcoma. —, alkaline phosphatase activity (μmoles of *p*-nitrophenyl phosphate hydrolyzed/min/l.

Zusammenfassung. In Yoshida-Sarkomzellen ist die alkalische Phosphatase vorwiegend in der partikelfreien Fraktion in gelöster Form lokalisiert. Gelfiltration führt zu einer Auftrennung des Enzyms im partikelfreien Überstand von 6 Fraktionen, des Enzyms in den Zellkernen bei 2 Fraktionen.

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